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Associations of the PTEN −9C>G polymorphism with insulin sensitivity and central obesity in Chinese

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ABSTRACT

Background: Phosphatase and tensin homolog on chromosome 10 gene (PTEN) is known as a tumor-suppressor gene. Previous studies demonstrated that PTEN dysfunction affects the function of insulin. However, investigations of *PTEN* single nucleotide polymorphisms (SNPs) and IR-related disease associations are limited. The aim of the present study was to investigate whether its polymorphism could be involved in the risk of metabolic syndrome (MetS).

Methods: The genotype frequency of PTEN −9C>G polymorphism was determined by using a Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) method in 530 subjects with MetS and 202 healthy control subjects of the Han Ethnic Chinese population in a case–control analysis.

Results: The PTEN −9C>G polymorphism was not associated with MetS or its hyperglycemia, hypertension and hypertriglyceridemia components. In the control individuals aged <60 years or ≥60 years, the CG genotype individuals had lower insulin sensitivity than CC individuals ($P < 0.05$). In the <60-year-old MetS group and normal glucose tolerance (NGT) subgroup, the CG individuals had lower insulin sensitivity and higher waist circumference (WC) and waist-height-ratio (WHtR) than CC individuals ($P < 0.05$). Multiple linear regression analysis showed that the *PTEN* polymorphism ($P = 0.001$) contributed independently to 4.2% (adjusted R^2) of insulin sensitivity variance (estimated by Matsuda ISI), while age ($P = 0.004$), gender ($P = 0.000$) and the PTEN polymorphism ($P = 0.032$) contributed independently to 5.6% (adjusted R^2) of WHtR variance.

Conclusions: The CG genotype of PTEN −9C>G polymorphism was not associated with MetS and some of its components as well. However, it may not only decrease insulin sensitivity in the healthy control and MetS in pre-elderly or NGT subjects, but may also increase the risk of central obesity among these MetS individuals.

1. Introduction

Metabolic syndrome (MetS) refers to a cluster of correlated complex disorders that include central obesity, hyperglycemia, hypertension, dyslipidemia, an increased risk of developing cardiovascular disease (CVD) and type 2 diabetes mellitus (DM) (Eckle et al., 2005). MetS is now commonly used in large-scale studies around the world and is considered as an emerging epidemic in developing Asian countries, including Singapore, China, Japan and Korea, with a prevalence of 8%–13% in men and 2%–18% in women, depending on the population and definitions used (Lee et al., 2007; Park et al., 2007; Thomas et al., 2005). Its exact pathogenesis has been poorly understood until now (Grundy et al., 2005), but insulin resistance (IR) from abnormal insulin receptors and insulin signaling is considered a common pathophysiological background of MetS. IR induces a decrease in skeletal muscle and adipose tissue glucose disposal and impairs hepatic glucose production

Abbreviations: PTEN, phosphatase and tensin homolog on chromosome 10; SNP, single nucleotide polymorphism; MetS, metabolic syndrome; NGT, normal glucose tolerance; IR, insulin resistance; CVD, cardiovascular disease; PD, prediabetes; DM, type 2 diabetes mellitus; BMI, body mass index; WC, waist circumference; WHtR, waist circumference/height; Matsuda ISI, Matsuda insulin sensitivity index; HOMA-IR, homeostasis model assessments of insulin resistance; HOMA-β, homeostasis model assessments of β-cell function; PI3K, phosphatidylinositol (PI) 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; MMAC1, multiple advanced cancers 1; TEP1, TGFβ-regulated and epithelial cell-enriched phosphatase 1; NAFLD, nonalcoholic fatty liver disease; OGTT, oral glucose tolerance test; HbA1c, glycated hemoglobin; MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry; ANOVA, analysis of variance; OR, odds ratio; CI, confidence interval.

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(Taniguchi et al., 2006), resulting in hyperglycemia and abnormal lipid metabolism. Insulin binds and phosphorylates its receptors at tyrosine residues and activated insulin receptors bind and activate phosphatidylinositol (PI) 3-kinase (PI3K). Activated PI3K leads to the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3), which is a key second messenger in various insulin metabolic effects (White, 2002). The signal is transduced to downstream protein kinase A (PKA) and/or protein kinase C (PKC) (Saltiel and Kahn, 2002), regulating gluconeogenesis, glycolysis and the metabolism of lipids and energy (Miyake et al., 2002).

Phosphatase and tensin homolog on chromosome 10 (PTEN), also known as mutated in multiple advanced cancers 1 (MMAC1) or TGFβ-regulated and epithelial cell-enriched phosphatase 1 (TEP1), is a tumor-suppressor gene located on chromosome 10q23.3. The *PTEN* gene contains nine exons and encodes a 403 amino acid protein, containing a PIP2 binding site, a phosphatase domain, a C2 domain with phosphorylation sites and a PDZ binding motif (PSD-95, Discs-large, ZO-1) from the N terminal to the C-terminal (Maehama et al., 2001). PTEN was initially identified as a tumor-suppressor in Cowden syndrome (Steck et al., 1997), and mutations and/or deletions of the *PTEN* gene have been found in many cancers including prostate cancer, hepatocarcinoma and endometrial carcinoma (Stambolic et al., 1998). PTEN is also associated with nonalcoholic fatty liver disease (NAFLD) and diabetes (Hamada et al., 2005; Yamada and Araki, 2001).

PTEN acts as a protein phosphatase and a dual-specificity lipid phosphatase. Activation of the protein phosphatase is a critical regulator for cell growth, survival, proliferation and migration (Stambolic et al., 1998), while activation of its dual-specificity lipid phosphatase antagonizes PI3K, thus acting as a negative regulator of the PI3K/PI(3,4,5)P3/Akt pathway that involves insulin. PTEN dephosphorylates phosphatidylinositol-3,4,5-triphosphate (PIP3), the main producer of phosphoinositide 3-kinase (PI3K), hindering PI3K signal transduction.

Although PTEN is associated with glucose and lipid metabolism, the association of its SNPs with metabolic diseases is rare. Indeed, while four *PTEN* polymorphisms were identified in Caucasian patients with type 2 diabetes, they were not associated with the disease (Hansen et al., 2001). Three different variants of *PTEN* were previously identified in Japanese diabetic patients, and the substitution of C with G at position −9 (−9C → G) located in the 5′-terminal untranslated region (UTR) of exon 1 was associated with type 2 diabetes. Transfection of *PTEN* with this SNP resulted in a significantly higher expression level of PTEN protein, leading to decreased phosphorylation of Akt in Cos1 cells and indicating that this *PTEN* polymorphism hydrolyses PI3-kinase products and prohibits insulin signaling, resulting in IR (Ishihara et al., 2003). As a modulator of insulin signaling, we considered *PTEN* to be a candidate gene for the development of IR and MetS. In this study, we evaluated the association of the PTEN −9C>G polymorphism with IR, MetS and its components in the Han ethnic group of China.

2. Materials and methods

2.1. Subjects

The 530 MetS participants studied were recruited from the Hangtian community of Chengdu, Sichuan province, China between September and November 2011. A cohort of 1200 individuals aged 40–70 years old were recruited from Yincao Community Hospital of Chengdu (Sichuan, China), and 202 of these were chosen as a healthy control group according to physical examinations and clinical biochemical investigations. Control subjects were from the Han ethnic group of Chengdu city and had no genetic relationship with the case group. A standard questionnaire was administered by trained staff to all individuals to obtain the following information: age, gender, address, employment details, smoking status, family history and disease status.

2.2. Diagnosis criteria

For the diagnosis of MetS, we followed the 2005 International Diabetes Federation consensus (Alberti et al., 2005) of a waist circumference ≥90 cm in men and ≥80 cm in women (for South Asians and Chinese) plus two or more of the following components: elevated serum plasma triglyceride (TG) (≥1.69 mmol/L [≥150 mg/dL]) or receiving current medication for high serum plasma TG; low plasma high-density lipoprotein-cholesterol (HDL-C) (<1.04 mmol/L [<40 mg/dL] for men, <1.29 mmol/L [<50 mg/dL] for women) or receiving current medication for low plasma HDL-C; hypertension (≥130/≥85 mm Hg) or previously treated hypertension; impaired fasting glucose (≥5.6 mmol/L [≥100 mg/dL]) or previously diagnosed type 2 diabetes.

Subjects were included in this study and classified into categories of glucose tolerance according to 2003 ADA criteria (Genuth et al., 2003): normal glucose tolerance (NGT) fasting plasma glucose (FPG) <5.6 mmol/L and 2 h PG <7.8 mmol/L; hyperglycemia including prediabetes (PD) and DM, PD: FPG ≥5.6 to <7.0 mmol/L and/or 2 h PG ≥7.8 to <11.1 mmol/L; DM: FPG ≥7.0 mmol/L and/or 2 h PG ≥11.1 mmol/L.

Subjects with the following conditions were excluded from the study: a history of cardiovascular events (according to medical documents of secondary or tertiary hospitals); receiving oral or intravenous corticosteroid hormone treatment; hepatic cirrhosis and ascites; hyperthyroidism or hypothyroidism; malignant tumor; severe disability or mental disorder; pregnancy or breast-feeding women.

Informed written consent was obtained from each participant and the study protocol was approved by the Biological Sciences Ethical Committee of the West China Hospital, Sichuan University, China.

2.3. Clinical and biochemical measurements

Each participant underwent a detailed clinical examination, a standardized oral glucose tolerance test (OGTT) and biochemical measurements.

Body weight (to the nearest 0.1 kg) and height (to the nearest 0.5 cm) were measured with subjects wearing light clothing without shoes. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Waist circumference (cm) was measured between the lowest rib and the iliac crest at the end of expiration with the subject in a standing position. Waist-to-height ratio (WHtR) was calculated by dividing waist circumference (cm) by height (cm). Blood pressure was measured three times at 5 min intervals in the sitting position after 15 min of rest, and the mean value was used.

After an overnight fast, 5 mL of blood were obtained from an antecubital vein without compression before 09:00 h. Each participant ingested a solution containing 75 g glucose at 08:00 h. Plasma samples were obtained before oral glucose loading and at 30 and 120 min during the OGTT. Plasma glucose levels were determined in duplicate by a glucose–oxidase method adapted to an automated analyzer (Hitachi 704, Boehringer Mannheim). Triglyceride, HDL-C and low-density lipoprotein-cholesterol (LDL-C) levels were determined by enzymatic methods with commercial reagent sets (Boehringer Mannheim). Serum insulin concentrations were measured by electrochemiluminescence (Cobas e411, Roche Company, Switzerland) and glycated hemoglobin (HbA1c) was determined by high performance liquid chromatography (HPLC, Bio-Rad D-10 hemoglobin A1C radiometer).

2.4. Islet B cell secretion function and IR assessment

IR and islet B cell secretion function were calculated by HOMA2 Calculator software (Wallace et al., 2004) (HOMA Calculator version 2.2; http://dtu.ox.ac.uk/homa). The HOMA2 model is a combination of HOMA formulation and computer technology to realize automatic computation. HOMA2-IR and HOMA2-%β represent IR and isletβ cell secretion function, respectively.